India needs more food to feed the present and the increasing population and the only way is to increase the per acre yield as there is no scope to extend the acreage under cultivation except for marginal adjustments. The use of pesticides will greatly contribute to this increased agricultural production and plant protection will become a routine practice on the farm. The use of pesticides in agriculture on a commercial scale was started in 1948-49 and their use increased gradually but greatly accelerated with the implementation of the grow more Food Schemes and the Five Year Plans. Indian Agriculture is in the throes of change. Agricultural education system has first to discern direction of the change and the environment leading to this change and then reorient and adapt itself to fasten the desired change (Mehta, 1999).

The production of pesticides in India started in 1952 with the establishment of a plant for the production of BHC near Calcutta, and India is now the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally (Mathur, S.C., 1999). There has been a steady growth in the production of technical grade pesticides in India, from 5,000 metric tons in 1958 to 1,02,240 metric tons in 1998. In 1996-97 the demand for pesticides in terms of value was estimated to be around Rs. 22 billion (USD 0.5 billion), which is about 2% of the total world market. The pattern of pesticide usage in India is different from that of the world in general. In India 76% of the pesticide used is insecticide, as against 44% globally (Mathur, S.C., 1999). The use of herbicides and fungicides is correspondingly less. The use of pesticides in India is mainly for cotton crops (45%), followed by paddy and wheat.

In the beginning, all the compounds synthesized were chlorine containing hydrocarbons or organochlorines (OCs). Later, organophosphates (OPs) and carbamate compounds were synthesized during 1945-1950. In 1960s, the synthetic pyrethroids of plant derivatives were synthesized and all these contributed greatly in
pest control and agricultural output. Ideally a pesticide must be lethal to the targeted pests, but not to non-target species, including man. Unfortunately, this is not, so the controversy of use and abuse of pesticides has surfaced. The qualitative and quantitative chemicals usage is of great concern ecologically. The discriminate use of chemicals is for the control of insect pests by elimination of target species whereas, indiscriminate usage posed the problem on non-target organisms including man (Koteswara Rao 2003).

In India, the first report of poisoning due to pesticides was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with parathion (Karunakaran, 1958). This prompted the Special Committee on Harmful effects of pesticides constituted by the ICAR to focus attention on the problem (Eds. Wadhwani and Lall, 1972). Numerous problems are associated with the effects on non-target organisms which are of concern; start with the occurrence persistence and metabolism of these chemicals in aquatic bodies (Cope 1964; Tarzwell 1963). Consequently, their residues began to appear in foodstuffs (Mukherjee et al., 1980; Passino, 1981). Rachel Carson (1962) vividly explained the adverse effects of indiscriminate use of pesticides in her book ‘Silent Spring’. The indiscriminate use of pesticides has manifested itself in the high mortality and reduced reproductive potential in organisms (Brown and Frank, 1980; Yockin et al., 1980) and resulted in the development of resistance to pesticide in both target and non-target species (Conway and Comins, 1981). In 2003, the EPA Environmental Fate and Effects Division reported that insecticide was the second most widely detected in surface water in the U. S. Geological Survey’s (USGS) National Water Quality Assessment (NAWQA) monitoring program. Certain environmental chemicals including pesticides termed as endocrine disruptors were known to elicit their adverse effects by
mimicking or antagonizing natural hormones in the body and it has been postulated that their long-term, low-dose exposures are increasingly linked to human health effects such as immunosuppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Crisp, et al., 1998; Brouwer, et al., 1999).

The undue persistence, high mammalian toxicity and developing resistance to the organochlorine, organophosphate, carbamate and synthetic pyrethroids insecticides led to a ban or restriction on their used, in many developed and developing countries. Hence, it has become imperative to investigate and synthesize new molecules which are proved to be having less mammalian toxicity, causing no resistance development and less persistent. In this process, several new generation pesticides were synthesized. A few among them are imidacloprid (nicotine derivative), spinosad (biotech compound) and indoxacarb (oxadiazine compound) (Brouwer, et al., 1999).

Carbamate insecticides are among the most toxic compounds employed for insect control. The need for a complete assessment of their potential toxicological hazards to man and domestic animals has assumed greater importance. Thiodicarb, a carbamate insecticide has been used for the last several years in agriculture. It was moderately toxic after oral administration and was classified as moderately hazardous by WHO.

Thiodicarb was reviewed for residues in 1985, and supervised field trial data for various crops were considered in 1987 and 1988.
**FIG.1.1. MOLECULAR STRUCTURE:**

![Molecular Structure of Thiodicarb](image)

**Chemical Characteristics:**

Thiodicarb is a white crystalline powder with a slight sulfurous odor. It has a melting point 173-174°C. Thiodicarb is stable in light and ambient conditions and unstable in alkaline conditions. Its main degradation product is methomyl.

**Table.1.1. CHEMICAL IDENTITY:**

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>IUPAC: 3,7,9,13-tetramethyl-5,11-dioxa-2,8,14-trithia-4,7,9,12-tetra-azapentadeca-3,12-diene-6,10-dione</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A</td>
<td>Dimethyl N,N'-thiobis[(methylimino)carbonyloxy]]bis(ethanimidothioate)</td>
</tr>
<tr>
<td>Chemical group</td>
<td>Carbamates</td>
</tr>
<tr>
<td>BSI common name</td>
<td>Thiodicarb</td>
</tr>
<tr>
<td>Synonyms</td>
<td>Nivral; Larvin; Chipco; Skipper; Semevin;</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C10H18N4O4S3</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>354.5</td>
</tr>
<tr>
<td>Formulation</td>
<td>WP 75%</td>
</tr>
<tr>
<td>Trade name</td>
<td>Larvin</td>
</tr>
</tbody>
</table>
Table I.2. PHYSICAL AND CHEMICAL PROPERTIES:

<table>
<thead>
<tr>
<th>Pure active ingredient</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance: White powder containing small aggregates</td>
<td>Robles and Bascou, 2000</td>
</tr>
<tr>
<td>Melting point: 172.6°C</td>
<td>Robles and Bascou, 2000</td>
</tr>
<tr>
<td>Decomposition point: 184.7°C</td>
<td>Robles and Bascou, 2000</td>
</tr>
</tbody>
</table>

**Hydrolysis:**

PH 5 (buffered, 25°C) DT50 = 78.4 days Feung and Weisbach, 1991b
PH 7 (buffered, 25°C) DT50 = 31.6 days
PH 9 (buffered, 25°C) DT50 = 0.48 days
Photolysis: DT50 = 7.6 days, (k=1.05 x 10^{-6} sec^{-1}) Feung and Blanton, 1987
Vapour pressure:

- 2.7 X 10^{-3} Pa at 25°C
- 6.9 X 10^{-4} Pa at 35°C

**Solubility:**

**Water**

- Deionised water (25°C) 22.19 μg/ml
- Water buffered (pH 3, 25°C) 26.88 μg/ml
- Water buffered (pH 5, 25°C) 29.83 μg/ml
- Water buffered (pH 7, 25°C) 24.47 μg/ml

I.3. Toxicological Effects:

I.3.1. Impact on Environment:

Pesticides contaminate soil, water and other vegetation. In addition to killing insects or weeds, pesticides are toxic to a host of other organisms including birds, fish, beneficial insects, and other non-target organisms including humans. Insecticides are generally the most acutely toxic class of pesticides, but herbicides can also pose...
risks to non-target organisms. Pesticides are found as common contaminants in soil, air, water and on non-target organisms in our urban landscapes. Once there, they can harm plants and animals ranging from beneficial soil microorganisms and insects, non-target plants, fish, birds, and other wildlife (USGS, 1995).

Available environmental fate studies show that, thiodicarb degrades rapidly into methomyl under most conditions. While the parent chemical does not appear to be very persistent or highly mobile, the degradate methomyl is more persistent, more mobile, and more toxic (Bortone et al., 1989). Thiodicarb rapidly degrades (half lives on the order of a few days) primarily by metabolism and hydrolysis in alkaline conditions. It may be more persistent under drier conditions. Methomyl appears to be moderately persistent and highly mobile in the environment. The dominant routes of dissipation are metabolism (biologically mediated degradation), leaching, and photolysis in clear waters.

Laboratory studies show that thiodicarb is practically non-toxic to birds but moderately to highly toxic to small mammals on an acute oral basis. Methomyl is highly toxic to birds and mammals on an acute oral basis but only slightly toxic to birds on a sub-acute dietary basis. Thiodicarb may result in chronic risks to certain species that frequent short grass (e.g., ducks, geese and swans). Methomyl, as a degradate, poses acute risks to birds and mammals that feed on short and tall grasses, broad leaf plants, and small insects.

**I.3.2. Ecological Effects:**

Environmental Protection Agency (EPA) is generally concerned about the ecological effects to terrestrial wildlife and aquatic organisms posed by exposure to thiodicarb. The risk assessment for thiodicarb and its degradate methomyl shows various levels of concern regarding avian risk and mammalian risk from multiple
applications of thiodicarb at short intervals. In addition, most agricultural uses present acute and chronic risks at varying levels to endanger and non-endangered aquatic organisms. The major concerns for non-target organisms are the chronic risks posed by the use of thiodicarb to non-target mammalian and freshwater invertebrate organisms. With risk mitigation measures in place, the Agency considers these risks are acceptable. Based on the reviews of the generic data for the active ingredient thiodicarb, the Agency has sufficient information on the health effects of thiodicarb and on its potential for causing adverse effects on fish, wildlife and the environment. The Agency has determined that thiodicarb products, labeled and used as specified in the Reregistration Eligibility Decision, will not pose unreasonable risks to humans or the environment.

Fish are continued to be an extremely reliable component of an aquatic monitoring system because they integrate the effect of detrimental environmental changes as consumers which are relatively high in the aquatic food chain. The fish as a bioindicator species plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to the changes in the aquatic environment. The sudden death of a fish indicates heavy pollution; the effects of exposure to sub-lethal levels of pollutants can be measured in terms of biochemical, physiological or behavioral responses of the fish (Howells, 1994). Fish are very good biosensors of aquatic contaminants. Fish were exposed to any toxicant of different concentrations, due to stress the physiology gets disturbed, thus considerably affecting the enzyme system. Fish represents the oldest and most diverse class of vertebrates, comprising around 48% of the known member species in the subphylum Vertebrata (Altman & Dittmer, 1972).
I.4. Significance of the Present Study:

Guntur district in Andhra Pradesh, India is predominantly an agricultural district located on the Western bank of the lower reaches of river Krishna (Lat. 15° 18'- 16° 50' North; Long. 70° 10'- 80° 55' East) traditionally, tobacco, cotton, chillies, black gram and green gram are cultivated in wetland regions. The dry land commercial crops entail heavy investments and yield good profits. Also, it is well known that all these are heavily sprayed crops. It is interesting to note that of the total annual sales of pesticides in 23 districts of Andhra Pradesh; nearly 30-40% of the total quantity is sold through various outlets in Guntur district alone, (Personal information...
from the joint director of Agriculture, Government of Andhra Pradesh, Guntur district). The commercial outlook of farmers in this region was mainly aimed at the maximization of the profit margin which in turn has resulted in heavy consumption of pesticides. In the recent past the local agriculture officers advise the farmers to reduce indiscriminate pesticide spraying and to abate the usage of banned insecticides. From the upland regions of this locality, the pesticides used are washed to the low land water bodies through surface runoff, where, the aquaculture activities are taken up by the farmers.

Aquatic toxicology is a multidisciplinary field which integrates toxicology, aquatic ecology and aquatic chemistry (Rand, Gary M et al, 1985). Some studies were aimed towards development of a behavioral assay for toxicity testing, based on changes in behavior such as courtship (Stafford and Ward, 1983; SchroÈder and Peters, 1988a; Bortone et al., 1989). This information could be used in a similar vein to lethal toxicity tests, but with behavioural responses as an end point (Stafford and Ward, 1983; Little et al., 1985; Bortone et al., 1989). SchroÈder and Peters (1988a) contended that as a behavioural assay, courtship by guppies (Poecilia reticulata, Poeciliidae) is extremely sensitive to very low concentrations of aquatic contaminants. Stafford and Ward (1983) found orange chromides (Etroplus maculatus, Cichlidae) also to be a suitable test organism and proposed that behavioural assays are liable to be more sensitive measures in effects of toxicants than previous methods.

Toxicity tests may be used to screen new chemicals, and to formulate water quality criteria and standards (Council of the European Communities, 1978; Alabaster and Lloyd, 1982; Abel, 1989; Lloyd, 1992; Howells, 1994). Standardized protocols for toxicity tests, for example LC$_{50}$ tests
using guppies or Daphnia magna (Daphniidae), or life-cycle tests using salmonids, are often used (Alabaster and Lloyd, 1982; Lloyd, 1992; Howells, 1994; Mason, 1996). Toxicity tests are also used to assess hazards presented by effluents, Example, from industry or sewage treatment works. Such monitoring of effluent is especially useful when substances are difficult to analyze or where a concoction of substances is present (Alabaster and Lloyd, 1982).

In addition to analytical testing for known pollutants, aquatic, whole effluent toxicity tests have been standardized and are performed routinely as a tool for evaluating the potential harmful effects of effluents discharged into surface waters (EPA). For the Clean Water Act under United States Environmental Protection Agency there are water quality criteria and water quality standards derived from aquatic toxicity tests (EPA). The presence of such compounds in organisms does not indicate a health risk as long as the levels are low and below toxicity thresholds. Most insecticides ultimately find their way into rivers, lakes and ponds (Tarahi Tabrizi, 2001; Honarpajouh, 2003; Bagheri, 2007; Shayeghi et al., 2007; Vryzas et al., 2009; Werimo et al., 2009; Arjmandi et al., 2010) and have been found to be highly toxic to non-target organisms that inhabit natural environments close to agricultural fields.

Different concentrations of insecticides are present in many types of wastewater and numerous studies have found them to be toxic to aquatic organisms especially fish species (Talebi, 1998; Üner et al., 2006; Banaee et al., 2008). Fishes are particularly sensitive to the environmental contamination of water. Hence, pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (Banaee et al., 2011). Authors found out those different kinds of insecticides can cause serious
impairment to physiological, health status of fishes and since fishes are important sources of proteins and lipids for humans and domestic animals, so health of fishes is very important for human beings (Begum, 2004; Monteiro et al., 2006; Siang et al., 2007; Banaee et al., 2009).

In order to have a thorough knowledge of the extent and type of damage caused by these insecticides, morphological and biochemical parameters should be established (Shakoori et al., 1976). Pesticides not only produce morphological or pathological changes but also cause significant bio-chemical alterations in the living system (Edwards, 1973; ‘O’Brien, 1977).

The present study has been undertaken to have a basic understanding of the acute toxicity and chronic effects of Thiodicarb (Larvin 75% WP) on freshwater fish *Labeo rohita*. The test fish were highly sensitive to the alterations in the quality of water. *Labeo rohita* was selected as the test species as it is highly consumed by the people of Guntur region. Ballesteros et al. (2011) reported that during the initial 24 h of exposure, insecticides may be transported into various tissues of fish; Bioaccumulation rate of selected pesticide in fish depends on the species, life stages, the amount of fat reservation in different tissues and diet of fish, chemical and physical properties of insecticides and the rate of water pollution.

1.5. BIOLOGY OF THE TEST FISH:

Rohu is a column feeder and prefers to feed on plant matter including decaying vegetation. Spawning season generally coincides with the southwest monsoon. Spawning occurs in flooded rivers. Fecundity varies from 2,26,000 to 2,794,000 depending upon the length and weight of the fish and weight

*Labeo rohita* (Hamilton) is the most important among the three Indian major carp species used in carp polyculture systems. In India, it has been transplanted into almost all riverine systems including the freshwaters of Andaman, where its population has successfully established. The species has also been introduced in many other countries, including Sri Lanka, Japan, China, the Philippines, Malaysia, Nepal and some countries of Africa.

**Fig.I.3. Labeo rohita**

<table>
<thead>
<tr>
<th>Kingdom:</th>
<th>Animalia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum:</td>
<td>Chordata</td>
</tr>
<tr>
<td>Class:</td>
<td>Actinopterygii</td>
</tr>
<tr>
<td>Order:</td>
<td>Cypriniformes</td>
</tr>
<tr>
<td>Family:</td>
<td>Cyprinidae</td>
</tr>
<tr>
<td>Genus:</td>
<td><em>Labeo</em></td>
</tr>
<tr>
<td>Species:</td>
<td><em>Rohita</em></td>
</tr>
</tbody>
</table>

The traditional culture of this carp goes back hundreds of years in the small ponds of the eastern Indian states. The success in induced breeding in 1957 and the assured seed supply thereafter was the major factor for the development of its culture in freshwater ponds and tanks. Its high growth potential, coupled with high consumer preference, have established rohu as the most
important freshwater species cultured in India, Bangladesh and other adjacent countries in the region. Considering its importance in the culture system, emphasis has also been given to its genetic improvement through selective breeding in India.

Hence, in the present study an attempt has been made to assess the effect of Thiodicarb (Larvin 75% WP) on the fish *Labeo rohita*. Sensitivity, behavioral changes, oxygen consumption, biochemical changes, histological changes and bioaccumulation studies were carried out. A careful perusal of the literature reveals not much of work has been done on the Acute and chronic affects of the selected thiodicarb, pesticide as an above mentioned disrupting chemical on the major carp *Labeo rohita*. 